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EVIDENCE OF DARK AVOIDANCE BY PHOTOTROPHIC PERIPHYTIC DIATOMS IN LOTIC SYSTEMS[1]

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ABSTRACT

Short-term (24–48 h) colonization dynamics of periphytic diatoms on artificial (styrofoam) substrata were examined using fast-flushing, continuous-flow troughs located on the North Thompson River, British Columbia. Two parallel troughs, one exposed to natural light and the other completely darkened, showed significant differences in periphyton biomass, chlorophyll a*, and algal taxonomic composition with 24 h. Experiments which commenced at the onset of natural darkness demonstrated that rates of algal immigration during the night were the same in both troughs. Within 2–3 h of sunrise, however, certain diatom species (most notably* Hannaea arcus *(Ehr.) Patr. and* Diatoma tenue *Ag.) selectively emigrated from the artificially darkened trough but remained in the trough exposed to natural light. More closely adhering species such as* Achnanthes minutissima *Kütz. also showed significant emigration from the darkened trough after light deprivation for two photoperiods. Data from adhesion, emigration, and sinking rate experiments indicate that differential egress of cells from the darkened versus the lighted environments is the result of cellular regulation of buoyancy or form resistance.*

Key index words: algal buoyancy; algal sinking rate; dark avoidance; diatoms; periphyton; species succession

In 1980–1981 nutrient-limited periphyton growth rate studies were conducted using an experimental, continuous-flow trough apparatus on the North Thompson River, British Columbia (Bothwell 1983, Bothwell and Jasper 1983, Bothwell 1985). During these experiments with parallel light and dark troughs, 24 h chlorophyll (Chl *a*) accrual rates were often 2–4 times greater in the light trough than the dark trough (Bothwell and Jasper 1983). This difference could not be explained by algal growth or pigment synthesis as the time frame was too short and water temperatures too low (1–3° C). Although shading within attached algal communities is thought to cause the loss of some diatom species (Patrick 1976, Hudon and Bourget 1983) the time frame for elimination of newly colonizing cells by light-limitation in the dark trough should have been much longer than was indicated by the rate of change in Chl *a*. These observations suggested the existence

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of a physiological mechanism whereby lotic periphyton species could move and thus avoid colonization of dark habitats. Movement could be achieved by algal cells being either less adhesive or more buoyant in the dark. Either mechanism could have significant implications for both competition and succession in developing periphyton communities. It could explain species replacement under different light regimes that would not be a direct result of competition for available light.

A series of experiments was therefore conducted at the same site between 1983–1986 to establish whether lotic periphytic diatoms are able to selectively leave darkened environments and to determine the physiological mechanism(s) responsible for rapid (<24 h) colonization of light versus dark habitats. These experiments included a series of short-term (24 h) colonization trials in which changes in colonizing species of light versus dark troughs were documented. Other experiments tested two hypothesized mechanisms which might explain a rapid preferential loss of cells from darkened substrata: (1) cells become more adhesive in the light, or (2) diatoms alter their buoyancy or form resistance resulting in a selective loss of cells from darkened environments.

MATERIALS AND METHODS

Experimental apparatus and sampling procedure. The trough apparatus and periphyton sampling procedures used in this study have been described in detail elsewhere (Bothwell 1983). In brief, the apparatus consisted of two identical parallel Plexiglas flumes (each 2 m × 19 cm) located on the bank of the North Thompson River in Kamloops, British Columbia (Fig. 1A, B). One trough was enclosed with a light-tight cover and left in complete darkness, and the other was covered with plateglass and exposed to natural sunlight, which was continuously recorded with a LICOR (Lincoln, Nebraska) integrating meter (LI-550B) with a quantum sensor (LI-190SB). River water was continuously pumped to a 200 L headtank feeding both troughs. Discharge to each trough was 47 $L \cdot min^{-1}$ and resulted in a water depth and velocity of ca. 1.0 cm and 50 $cm \cdot s^{-1}$, respectively. Hydraulic residence time of the headtank was approximately 2 min and of the troughs, 4 s. Sheets of open-cell styrofoam-DB (Snow Foam Inc., El Monte, California) lined the bottom of both troughs and served as a microbial colonization substratum.

The substratum was sampled for Chl *a* and cell numbers by removing 5.0 cm^2 cores with a corkborer (Fig. 1C). Four replicate cores were routinely sampled for chlorophyll, and 4–10 cores were taken for cell counts. Samples were first removed 15 cm from the outlet of each trough, and subsequent samples were removed immediately adjacent to and upstream of the previous coring location. The total length of trough substratum sampled during any one experiment was usually less than 20 cm. The trough-substratum system has yielded highly replicable biomass estimates (Chl *a*: mean cv = 10.9% for quadruplicate cores collected on 115 separate occasions) (Bothwell 1983).

Short-term colonization experiments. Five periphyton accumulation experiments were conducted during January–April, 1983. All trials commenced after onset of darkness by placing fresh styrofoam in the troughs and starting water flow, which allowed both troughs to colonize at night for 12–14 h before sunrise. After commencement of water flow in darkness, 4 cores of the substratum were removed from each trough at 3–6 h intervals for Chl *a* determination. Chl *a* samples were extracted immediately in 90% acetone with a Polytron grinder. Analysis was made fluorometrically (Holm-Hansen et al. 1965) using a Turner Designs fluorometer equipped with a far-red sensitive photomultiplier (R446), a Corning CS-5-60 excitation filter and the combination emission filter system, Corning CS-2-64/Wratten-70, suggested by Loftus and Carpenter (1971). The ratio of fluorometric readings before and after acidification (chlorophyll acid ratio, CAR) was used to correct for phaeopigments and was used as a relative indicator of changes in community pigment composition.

Core samples for live diatom enumeration were removed from each trough at 6–12 h intervals. The adhering periphyton community was fragmented in deionized water with a Waring blender (10 s). Checks were made to ensure that the fragmentation procedure did not result in cell breakage. Then 5 or 10 mL of the resulting cell suspension was enumerated in Utermöhl settling chambers using the acid-fuchsin procedure of Owen et al. (1979). A minimum of 100 individuals of the dominant species and at least 500 cells in total were counted. Cell numbers were converted to cell volumes for each species using the mean dimensions of 10 specimens and planimetric determinations of the valve surface area of each taxon from drawings of Patrick and Reimer (1966, 1975).

During two experiments in February 1983, 1 L water samples were collected from the headtank at 1800, 2100, 2400, and 0600 h for determination of diatom cell numbers. Taxonomic composition of the river community passing through the troughs was compared to the colonizing community. Also during these two trials, Chl *a* in the river water during the night colonization period and Chl *a* of the colonizing community on the styrofoam was size-fractionated by passing subsamples through 53 μm Nitex netting. Concentrations of Chl *a* > 53 μm were determined by subtracting the amount of pigment passing through the net from total (retained by Whatman GF/F) Chl *a* values.

Neutral red experiment. To provide additional evidence of cell emigration, in one experiment cells colonized on the substratum were marked in situ with neutral red. This dye is a vital (sublethal) stain often used in 2–200 ppm concentrations as an indicator of viability for both plant and animal cells (Crippen and Perrier 1974, Harrison 1988). Preliminary tests with laboratory batch cultures of the diatom *Fragilaria vaucheriae* (Kütz.) Peters isolated from the Thompson River showed that neutral red (10 ppm exposure for 1 h) allowed easy distinction of living cells without affecting cell division rates.

Live cells which had settled overnight (14–15 February) in both troughs were labelled before sunrise with neutral red (Sigma Chemical Co.). This was done by continuous addition of an aqueous solution of dye for 1 h (0630–0730 h) producing a concentration of 10 ppm in the troughs. After cells in the troughs had been labelled, 10 cores of substratum were removed from each trough, fragmented, and quantitative counts of the resulting diatom suspensions were made in settling chambers. A total of 500 cells was counted from both light and dark troughs. Initial counts $\cdot m^{-2}$ at 0730 h were compared to counts made 4.5 h and 8.5 h later.

Substratum inversion experiments. During two trials, an attempt was made to determine if diatoms which settled on styrofoam during the night became more firmly attached to the substratum when exposed to light the following day. These tests were made before sunrise and at several later times during the daylight period. Eight replicate cores were carefully removed from the light trough. Four were directly extracted in 90% acetone for a total Chl *a* measure, while the remaining four were gently inverted and lowered into a beaker of deionized water. Using forceps, the core was then very lightly tapped three times to dislodge cells. After a 10 s pause, the cores were re-inverted and extracted in acetone to determine the remaining Chl *a*. Percent Chl *a* retained on the core was used as a crude measure of degree of adhesion of algal cells. Quantitative counts of diatoms were also made on

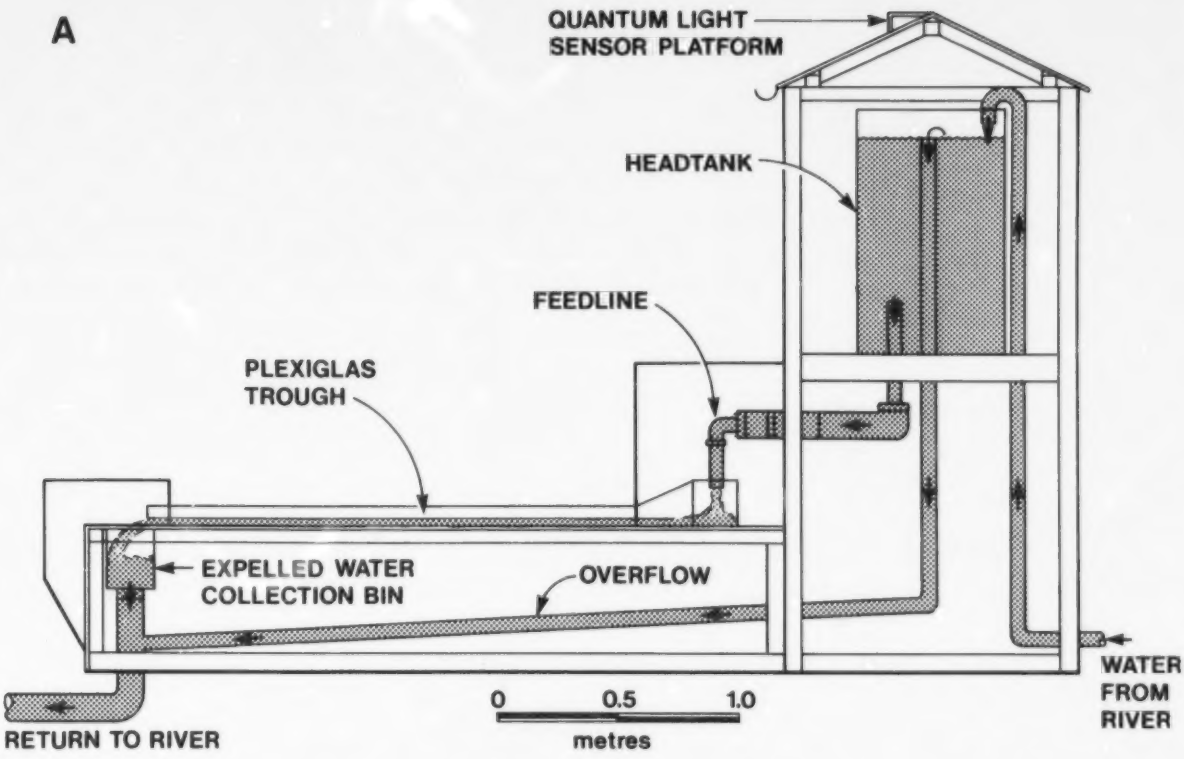

FIG. 1. A. Longitudinal section of trough apparatus. Solid arrows represent one-way flow of water through the system. Redrawn from Bothwell 1983. B. Parallel light (covered with plateglass) and dark troughs in operation. C. Close-up view of trough styrofoam substratum with cores removed for chlorophyll analysis and cell counts.

replicate cores before and after the inversion procedure to estimate the relative efficiency with which different species were dislodged. The retention of periphyton on the cores was statistically examined using the Student-Newman-Keuls multiple comparison test.

Water velocity experiments. In March 1986, experiments were conducted to determine how water velocity in the troughs influenced egress of cells during the day. For the first trial (4–5 March), both light and dark troughs were allowed to colonize overnight under standard hydraulic conditions used in all previous experiments; i.e. discharge, 47 $L \cdot min^{-1}$; velocity, 50 $cm \cdot s^{-1}$; depth, 1 cm. Following colonization, cell numbers in both troughs were determined at hourly intervals from 0600–1100 h. This run was repeated the next day but with one change. At 0630 h, discharge to each trough was reduced to 7 $L \cdot min^{-1}$, and water depth was increased so that velocity slowed to 3 $cm \cdot s^{-1}$. These changes in velocity and depth shifted flow in the troughs from super to subcritical conditions (Streeter 1962). Cell abundance was determined hourly following this change.

Sinking rate experiment. The sinking rates of diatoms settled in

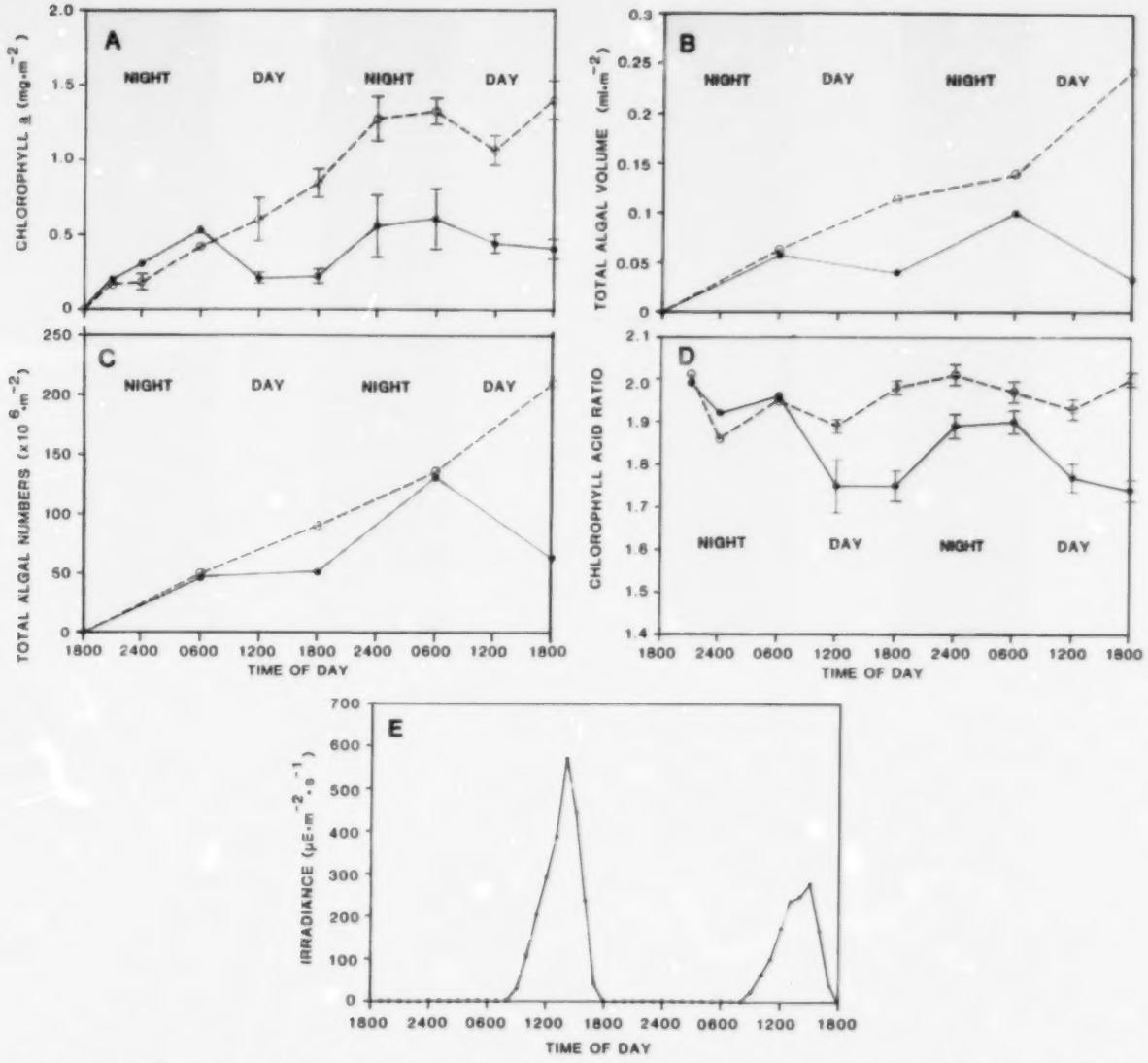

FIG. 2. 48 h time-course colonization experiment in continuous-flow light (– – –) and dark (——) troughs, 1–3 February 1983. A. Chlorophyll *a* with 95% confidence limits. B. Total algal cell volume. C. Total algal cell numbers. D. Chlorophyll acid ratio (fluorometric) with 95% confidence limits. E. Solar irradiance.

light and dark troughs were determined on 6 March 1986 using the SETCOL method and apparatus described by Bienfang (1981a). After allowing night time colonization of both troughs at flows of 47 L·min^{-1}, discharge was reduced to 7 L·min^{-1} to limit cell loss. Sinking rate experiments were conducted on algal samples collected from both light and dark troughs before sunrise (0600 h) and later in the morning (0930 h). Periphyton on the substratum was resuspended in filtered river water by vigorously agitating the styrofoam in a 2 L widemouth polyethylene container. The homogeneous cell suspension was then poured into water-jacketed SETCOL apparatus (Bienfang 1981a) and allowed to settle for 2–3 h at in situ river temperature. Samples from the light trough were incubated in columns illuminated with cool-white fluorescent light (100 μE·m^{-2}·s^{-1}), and samples from the dark trough were incubated in columns in total darkness. Diatoms were enumerated in Utermohl settling chambers using the same technique and procedures described above for samples from the troughs. Sinking rates were calculated based upon the change in vertical distribution of cells after a given time using the equations of Bienfang (1981a). Changes in sinking rate were interpreted as changes in buoyancy (cell density), although alteration of form resistance (both morphological and vital components) might also be involved (Reynolds 1984).

RESULTS

Short-term colonization experiments. Algal periphyton was completely dominated by diatoms. Macroinvertebrate grazers were not present. During the first 12 h (night) of the experiments, Chl *a* increased at the same rate in both light and dark troughs so that by morning, levels in the two were not significantly different (*t*-test, $P > 0.05$). Four hours after sunrise,

TABLE 1. *The dark-avoidance behavior found in the dominant lotic periphyton diatom taxa listed in descending order of response. Shown is the mean % change in live cell numbers in the light and dark troughs between sunrise and sunset during five replicated 24 h colonization experiments in 1983. Initial counts at dawn were made following 12–14 h of colonization during natural night time darkness. Final counts were made after sunset, 10–12 h later.*

	Mean % change during a single daylight period		Comparison of light vs. dark changes
	Light trough	Dark trough	
Hannaea arcus (Ehr.) Patr.	+72.0	−45.0	$P < 0.001$
Diatoma tenue Ag.	+76.5	−12.5	$P < 0.01$
Fragilaria vaucheriae (Kütz) Peters	+89.3	+25.3	$P < 0.05$
Gomphonema olivaceum (Lyngb.) Ag.	+91.7	+75.0	NS
Achnanthes minutissima Kütz	+59.7	+71.0	NS

however, Chl *a* levels in the light and dark troughs always differed significantly ($P < 0.01$) (Fig. 2A, E), which confirmed findings from earlier experiments on the North Thompson River (Bothwell and Jasper 1983). Divergence in Chl *a* values between the two troughs was the result of a decline in Chl *a* in the dark trough coupled with an increase in Chl *a* in the light trough (Fig. 2A). The temporal pattern of increased Chl *a* during the night followed by decreased levels during the day was observed in the dark trough for two consecutive diel cycles and roughly paralleled changes in algal biomass estimated from cell biovolumes (Fig. 2B).

Although Chl $a \cdot m^{-2}$ and cell volume $\cdot m^{-2}$ always declined in the dark trough during the first blocked photoperiod (Fig. 2A, B), total cell numbers $\cdot m^{-2}$ did not always decrease (Fig. 2C). The decline in algal biomass in the dark trough was associated with a sharp decrease in certain large-celled species during the first blocked photoperiod. Most conspicuous and consistent in this behavior was *Hannaea arcus*; in all experiments when *H. arcus* was numerically abundant, it averaged a 45% decline during the first 8 h light period (Table 1). In two of three experiments when *Diatoma tenue* was abundant, it exhibited the same pattern, demonstrating an average decline of 12.5% in 8 h (Table 1). Although numbers of *Fragilaria vaucheriae* in the dark trough increased rather than decreased in the first dawn-to-dusk period of three experiments, these increases were significantly less than in the light trough, suggesting a preferential egress of cells under artificially darkened conditions (Table 1). Two other taxa abundant during some trials, *Achnanthes minutissima* and *Gomphonema olivaceum*, did not exhibit dark trough declines in the first photoperiod (Tables 1, 2). Both, however, showed significant declines in the dark trough during the second daylight period of the 48 h trial (Table 2). Hence, all five numerically-dominant species demonstrated, in varying degrees, lower cell numbers following daylight deprivation.

Another consistent feature in all short-term colonization experiments was the presence of diel shifts in the Chl acid ratio (CAR). CAR declined during the day and increased again at night (Fig. 2D). The diel rhythm in CAR was much more pronounced in the dark trough compared to the light trough (Fig. 2D) and reflects the major changes occurring in species composition in the dark trough during each photoperiod.

Neutral red experiment. The neutral red experiment using the stained cells as tracers provided corroboration that differences in cell numbers between troughs after a daylight period were due to increased cell emigration from the dark trough. Additionally, this experiment provided evidence that the majority of *H. arcus* and *D. tenue* cells which will leave the dark trough are gone within 4.5 h of sunrise. In the dark trough, 86.4% of stained cells present at 0730 h had emigrated by 1200 h and, of those remaining, a further 48.0% were gone by 1600 h (a total loss of 93% in 8.5 h) (Table 3).

In contrast, the emigration rate of stained cells from the light trough was fairly uniform throughout the day. In the first 4.5 h (0730–1200 h), 26.6% of stained cells were lost, while a further 26.9% of those cells remaining at 1200 h were lost by 1600 h (Table

TABLE 2. *Numbers of live cells × $10^6 \cdot m^{-2}$ ± 95% C.L. of counts for species contributing >7% of the total cells counts at different times during a 48 h experiment (1–3 Feb. 1983) in continuous-flow light and dark troughs. Initial cell counts were made following 12 h of colonization during natural night time darkness. % change was computed as the difference between counts at sunrise and sunset of each photoperiod.*

	0600 h		1800 h		% Change	
	Light trough	Dark trough	Light trough	Dark trough	Light trough	Dark trough
First photoperiod						
Hannaea arcus	26.6 ± 3.2	22.9 ± 2.9	45.0 ± 5.9	16.8 ± 3.4	+69	−27
Diatoma tenue	7.5 ± 1.7	5.4 ± 1.4	9.7 ± 2.5	6.0 ± 1.7	+29	+11
Fragilaria vaucheriae	3.3 ± 1.2	3.8 ± 1.1	7.1 ± 2.3	3.7 ± 1.4	+115	−3
Gomphonema olivaceum	2.5 ± 1.1	4.8 ± 1.4	6.1 ± 2.1	7.5 ± 1.9	+144	+56
Achnanthes minutissima	4.3 ± 1.3	5.8 ± 1.4	8.3 ± 2.5	12.7 ± 2.3	+93	+119
Second photoperiod						
Hannaea arcus	54.2 ± 7.6	31.4 ± 5.7	92.0 ± 11.9	5.2 ± 1.6	+70	−83
Diatoma tenue	13.9 ± 3.8	16.8 ± 4.2	25.6 ± 6.6	4.4 ± 1.5	+84	−74
Fragilaria vaucheriae	15.0 ± 4.1	12.4 ± 3.9	24.4 ± 6.7	13.6 ± 2.6	+63	+10
Gomphonema olivaceum	15.6 ± 4.1	23.3 ± 5.1	16.0 ± 5.3	11.0 ± 2.4	+3	−52
Achnanthes minutissima	22.4 ± 4.8	31.0 ± 5.9	23.3 ± 6.4	18.4 ± 2.9	+4	−41

TABLE 3. *Neutral red experiment (14–15 Feburary, 1983). Numbers of diatom cells (× $10^6 \cdot m^{-2}$) stained with neutral red, unstained (newly appearing) cells, and total live cell counts following a 12 h colonization period during natural night time darkness and a 1 h exposure to 10 ppm neutral red before sunrise. Cell numbers at 0730 h (the initial count) are compared with counts later during the day at 1200 h and 1600 h. Also shown is the % change in cell numbers by 1200 and 1600 h from the initial count at 0730 h.* Hannaea arcus *and* Diatoma tenue *comprised 75–80% of the initial total cell numabers in both light and dark troughs.*

	0730 h (initial count)	1200 h (4.5 h later)	% Change	1600 h (8.5 h later)	% Change
Stained cells					
Light trough	17.7	13.0	−26.6	9.51	−46.3
Dark trough	14.6	1.98	−86.4	1.03	−93.0
Unstained (new) cells					
Light trough	—	6.4	+36.2[a]	10.6	+59.9[a]
Dark trough	—	5.0	+34.3[a]	6.0	+41.1[a]
Total live cells					
Light trough	17.7	19.4	+9.6	20.1	+13.6
Dark trough	14.6	6.98	−52.2	7.0	−52.0

[a] Unstained cells/total live cells at 0730 h × 100.

3). At the same time, total numbers of cells (stained plus new unstained cells) in the light trough slowly increased from 17.7 to 20.1 × $10^6 \cdot m^{-2}$ between 0730 and 1600 h (Table 3).

Losses of stained cells from the light trough presumably represent the rate of cell turnover under natural light conditions. The loss rate of about 6% · h^{-1} in the light trough, however, seems high even in these rapidly flushed systems. Indeed, total cell numbers increased by only 13.6% in the 8.5 h daylight interval (Table 3), much lower than the >70% average increase observed in all other experiments (Table 1). This difference suggests that the neutral red treatment interfered in some unknown way with the ability of cells to remain in the troughs. Numbers of newly appearing unlabelled cells in the light trough during the daylight period represent an increase of ca. 60% over cells present and stained before sunrise. Hence, it seems likely that the modest increase in light trough total cell numbers during the neutral red experiment was due to an abnormally high egress of stained cells.

In spite of possible interference by neutral red, differences in egress between light and dark troughs are striking and show that nearly 60% of cells that emigrated from the dark trough in the 4.5 h morning interval did so because they were not exposed to morning sunlight (Table 3). Since sunrise did not occur until after 0730 h on that date, the period of light deprivation required to produce this response was <5 h.

Current velocity experiments. Assuming that the rate of cell loss from the troughs is in part a function of the degree of shear stress, then lower water velocity should reduce cell egress. This was confirmed during the March 1986 trials. Results with water velocity set a 50 cm·s^{-1}, corroborated the results of all

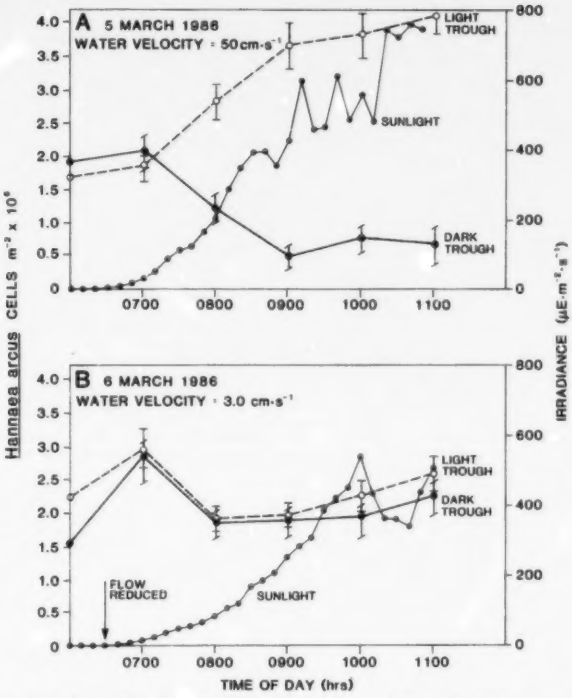

FIG. 3. Cell numbers of *Hannaea arcus* between 0600–1100 h in the light (dashed line) and dark (solid line) troughs following overnight colonization at a discharge of 47 L·min^{-1} and a water velocity of 50 cm·s^{-1}. (A) 5 March 1986, discharge and velocity maintained at initial colonization settings. (B) 6 March 1986, discharge and velocity reduced to 7 L·min^{-1} and 3 cm·s^{-1}, respectively, at 0630 h. Also shown is the solar irradiance on both days. Error bars are ±1 SD.

previous experiments and showed that between 0600–1100 h numbers of *Hannaea arcus* dramatically declined in the dark trough but increased in the light trough (Fig. 3A). In contrast, when discharge was reduced to 7 L·min^{-1} and velocity slowed to 3 cm·s^{-1}, there was no significant net loss of *H. arcus* from the dark trough (Fig. 3B). Flow reduction not only restricted cell loss from the dark trough but also stopped cell number increases in the light trough (Fig. 3B). Since it is unlikely that decreased water velocity would immediately affect rates of cell division, the major factor causing cell increases in these short-term experiments must be immigration, not cell division. Immigration rate is a function of water volume passing over the rough textured trough substrata, and reduction from 47 to 7 L·min^{-1} significantly lowered the colonization rate.

The hourly sampling intervals during the current velocity experiments also allowed a better estimate of the time required for dark avoidance to occur. These data indicate that the dark avoidance response neared completion within 2–3 h of sunrise (Fig. 3A). More importantly, the response was initiated after only a 1 h delay in morning light; by 0800 h cell numbers between the two troughs were significantly different ($P < 0.05$) (Fig. 3A).

TABLE 4. *Selective settlement of diatom species and Chl* a *on trough substrata. Mean % of total live diatom counts of the major taxa suspended in river water during night time colonization (1800, 2100, 2400, and 0600 h) is compared with the community composition in the troughs the following morning (0600–0730 h). Also shown is the Chl* a *retained by a 53 μm Nitex net expressed as a % of the total Chl* a *for communities suspended in the river water and settled in the troughs. Data presented are the mean results from two replicated 24 h experiments in 1983.*

	Cell size (μm^3)	% by cell numbers: Suspended in river water	% by cell numbers: Settled in trough	Trough: river water ratio[a]
Hannaea arcus (Ehr.) Patr.	1900	8.0	39.8	4.97
Diatoma tenue Ag.	180	21.3	14.9	0.70
Fragilaria vaucheriae (Kütz) Peters	340	12.8	12.9	1.01
Gomphonema olivaceum (Lyngb.) Ag.	480	13.3	8.4	0.63
Achnanthes minutissima Kütz	70	23.1	14.7	0.64
Chl *a* (>53 μm)		32.0	74.0	—

[a] Ratios > one indicate preferential immigration relative to other diatom species.

Size selective settlement. The different taxonomic composition of diatom communities and Chl *a* size distributions in river water during the night compared with the colonizing community in the troughs the following morning provides evidence for size selectivity of immigration. During two separate experiments, Chl *a* retained by a 53 μm net accounted for only 32.0% of total Chl *a* suspended in the river versus 74.0% of Chl *a* settled in the troughs the following morning (Table 4). The diatom showing the most pronounced selective settlement, *Hannaea arcus*, was by far the largest (1900 μm^3). It averaged only 8% of cell numbers of the community suspended in the river during the night, yet by dawn accounted for nearly 40% of the periphyton community (Table 4). This *H. arcus*-dominated size fraction had a higher (1.83–2.07) CAR value than the <53 μm size fraction (1.57–1.61). Hence, in the dark trough, night time settlement by *H. arcus* followed by selective egress of these cells during the day resulted in an augmented diurnal rhythm in CAR (Fig. 2D).

Diatom adhesion. Inversion experiments with colonized substrata from the light trough failed to show significant differences (SNK multiple comparison test, $P > 0.05$) in the degree of adhesion of chlorophyll-bearing organisms between pre-dawn (0630 h) and after sunrise measurements (0900, 1130, and 1500 h) in either of two replicated trials (Table 5). That is, longer exposure to light did not appear to increase cell adhesion to the substratum.

This conclusion was corroborated by a comparison of diatom cell counts on styrofoam cores before and after inversion. On a percentage basis, cells present in troughs at 1130 h did not show a greater tendency to adhere to the light-exposed substratum (Table 6). In fact, although there were no differences between adhesion in the light and dark for *Diatoma tenue*, *Fragilaria vaucheriae*, or *Achnanthes minutissima*, the data suggest that *Hannaea arcus* had higher adhesion in the dark than in the light trough.

TABLE 5. *Substratum inversion experiments. Student-Newman-Keuls multiple comparison test of the % chlorophyll* a *remaining on styrofoam cores from the light trough following the inversion-dislodgement procedure at different times of the day during two 24 h trials. Numbers underscored by the same line are not significantly different (P > 0.05). NA = not available.*

Date	Time of day (h): 0630	0900	1130	1500
17 Mar 1983	44.5	32.6	30.6	NA
14 Apr 1983	35.2	29.9	39.4	35.9

Diatom sinking. Although sinking rate per se has little relevance in shallow lotic systems, factors that influence sinking rate, such as buoyancy and drag, are also important in determining the settlement and resuspension of periphytic algae. Sinking rates for *Hannaea arcus* and *Diatoma tenue* declined during prolongation of the natural dark period (Fig. 4). Although sinking rates of *D. tenue* were slightly higher in the dark than the light trough between 0620–0900 h, buoyancy in both *D. tenue* and *H. arcus* tended to increase in the dark trough. Sinking rates in the dark actually became slightly negative by 0950–1230 h for both species (Fig. 4). At the same time that sinking rates decreased because of prolonged darkness, sinking rates increased in light-exposed cells. This combination accentuated total differences in sinking between cells in the light and dark troughs.

Sinking rates of *Fragilaria vaucheriae* did not follow this pattern. Whereas, sinking rates were higher in the dark than the light trough at 0620–0900 h, differences decreased as the length of light deprivation increased, primarily because sinking rates in the light trough declined during the course of the morning (Fig. 4). Other diatom species were present in too few numbers for statistically acceptable accuracy.

When the difference in sinking rates between cells in the light and dark troughs was positive, cells should have higher wash out from the dark trough relative to the light trough. By late morning (0950–1230 h) the sinking rate differential (light minus dark) was positive for all three species (Fig. 5). Moreover, the magnitude of this sinking rate difference ranked the three species in the same relative sequence for dark

TABLE 6. *Substratum inversion experiment. % cell numbers remaining on the styrofoam cores following the inversion and dislodgement procedure at 1130 h (14 April 1983) for the light and dark troughs.*

	Light trough	Dark trough
Hannaea arcus	24.5	66.3
Diatoma tenue	59.4	67.5
Fragilaria vaucheriae	43.8	29.5
Achnanthes minutissima	76.2	132.0[a]

[a] Number estimated after inversion was higher than pre-inversion although this was not significant (see Table 7).

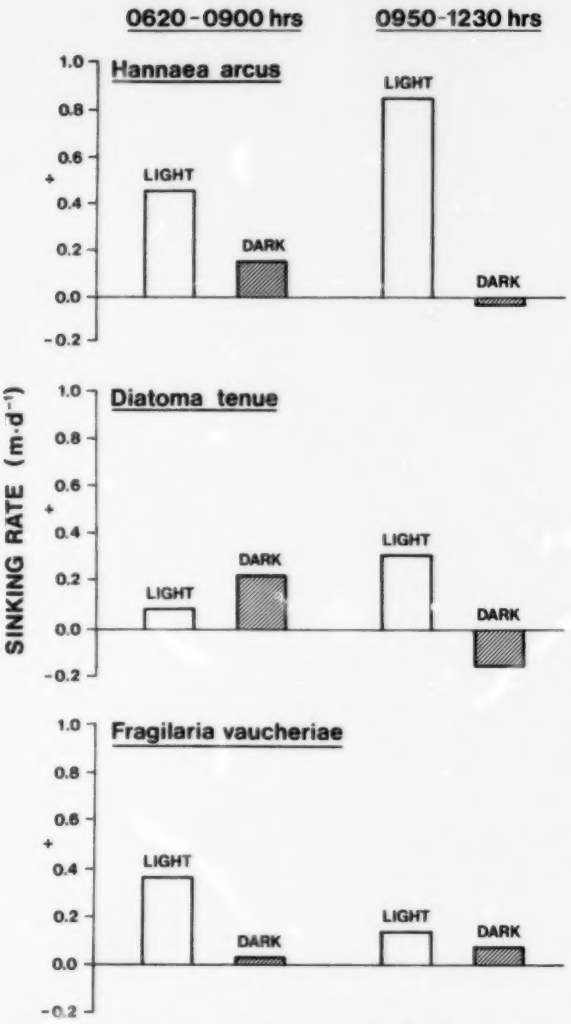

FIG. 4. Sinking rates of *Hannaea arcus*, *Diatoma tenue*, and *Fragilaria vaucheriae* measured between 0620–0900 h and 0950–1230 h on 6 March 1986 using SETCOL. Samples collected from the light trough are compared with samples collected from the dark trough.

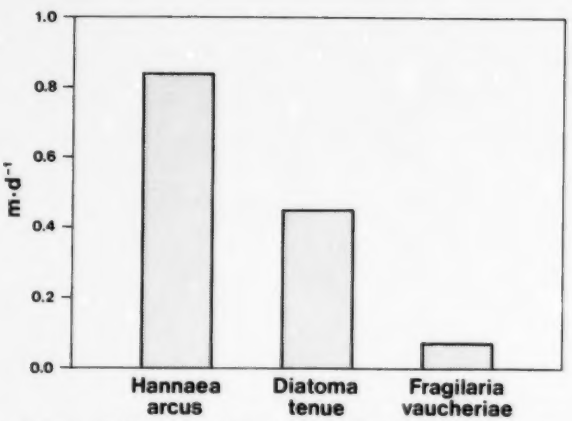

FIG. 5. Difference in SETCOL measured sinking rates between light and dark trough samples for the three major diatom species at 0950–1230 h on 6 March 1986.

avoidance response that was observed during short-term colonization experiments in 1983, i.e. *Hannaea arcus* > *Diatoma tenue* > *Fragilaria vaucheriae* (Fig. 5; Table 1).

DISCUSSION

In periphyton accumulation experiments, cell numbers·m^{-2} at any point in time represent the net result of incremental and decremental processes. Since diatom growth rates in the North Thompson River during winter are low (<0.2 div·d^{-1}) (Bothwell 1983, 1985), rapid biomass increases on an initially bare substratum are almost totally the result of immigration (settlement). Likewise, because of the absence of grazer populations, cell losses during initial colonization in rapidly flowing lotic systems are largely due to emigration (wash out). Therefore, in short-term (<24 h) colonization experiments, a decline in cells·m^{-2} could result from a decrease in immigration rate as well as an increase in emigration rate. The settlement rate of diatoms in transit through the dark trough during the day could not be altered relative to the light trough because the hydraulic residence time in most of these experiments was only 4 s. Declines in dark trough diatom biomass observed in all of these trials were the result of a higher emigration rate in cells which settled during the night and were not exposed to sunlight the following morning.

There are two plausible explanations for differential emigration of diatoms from light and dark troughs. The first is that cells may become more adhesive on exposure to light, perhaps through the photosynthetic production of extracellular polysaccharides (Hellebust 1974). Better adhesion to substratum by cells would reduce wash out in the light trough relative to the dark trough. The hypothesis of increased adhesion in the light, however, is not supported by data from the substratum inversion experiments. No significant differences were observed between pre-dawn and mid-day chlorophyll retention on cores from the light trough. Diatom cell counts also did not indicate any greater tendency for cells in the light trough to adhere to substrata compared to those in the dark trough. The data even suggested that the reverse might be true for one species.

The second possibility is that cells may change their density or alter their form resistance and the resulting shifts in buoyancy and drag, respectively, would facilitate differential erosion and egress of cells from the light and dark troughs. Because we are unable to distinguish between these two mechanisms we refer to them collectively as the "buoyancy-shift" hypothesis, with the understanding that changes in form resistance may also play a role.

A buoyancy-shift mechanism is consistent with the experimental observations reported here. First, since most cells that settle in the troughs fall into cavities in the open-cell matrix of styrofoam, factors which alter cell buoyancy will effect both settlement on and dislodgement from the substratum. A comparison of size distribution of chlorophyll-bearing organisms in the river during night with the size-spectrum on the styrofoam substratum in the troughs the following morning showed that the troughs were initially colonized by larger-sized (faster sinking) cells. The fact that larger-sized cells, i.e. *H. arcus*, which preferentially settled in the troughs during the night were also the ones which preferentially emigrated during prolonged darkness suggests that a buoyancy-shift was likely the mechanism responsible for selective losses of this species from the dark trough. Second, observations from the substratum inversion experiment also suggest that egress of cells from the dark trough could be easily explained by higher cell buoyancy in the dark than in the light. Cell numbers of all major diatom taxa in the dark trough at 1130 h before inversion were not significantly different from those on the light trough substrata following inversion (Table 7). In other words, cells which were prone to egress from the dark trough because of prolonged darkness were roughly equal in numbers to cells which would simply fall off an inverted substratum. Hence, only non-adherent cells appear to be susceptible to loss by egress from the dark trough. This observation may even explain the apparent anomaly of greater adhesion by *H. arcus* in the dark than in the light trough during the substratum inversion experiment. By 1130 h, cells of *H. arcus* as well as *D. tenue* and *F. vaucheriae* which were going to exit from the dark trough had already done so; cell numbers in the dark trough at 1130 h were significantly lower than in the light trough for all three of these species (Table 7). Therefore, *H. arcus* cells remaining in the dark trough at 1130 h were probably more firmly attached to the substratum than those in the light trough and were thus more resistant to dislodgement either by wash out or by inversion.

It is well known that buoyancy in planktonic algae is controlled by physiological mechanisms. Several workers have demonstrated diurnal rhythms in algal sinking rate in cultures grown under light : dark cycles (Eppley et al. 1967, Anderson and Sweeney 1977, Bienfang 1981b). Anderson and Sweeney (1977, 1978) found a diurnal rhythm in sinking rate, with maximum buoyancy (i.e. minimum sinking rate) occurring at the end of the dark period. The diel variation they observed was small, ca. 15–20%. On the other hand, Bienfang (1981b) found large (ca. 3 to 10-fold) diurnal variations in sinking rate using nutrient-limited cultures of the coccolithophorid, *Cricosphaera carterae*. Sinking rate reached a minimum at the end of the light period then steadily increased during the night. Granata (1987) reported large diel changes in sinking rates of the marine diatom *Coscinodiscus concinnus* grown under a 12:12 h LD regime, with sinking rates decreasing during darkness and increasing during light, i.e. the same timing and direction of diel sedimentation shifts found by Anderson and Sweeney (1977, 1978).

TABLE 7. *Substratum inverson experiment. Student-Newman-Keuls multiple comparison test of species cells numbers ($\times 10^6 \cdot m^{-2}$) on styrofoam cores at 1130 h on 14 April in the light and dark trough before and after the substratum inversion procedure. Numbers underscored by the same line are not significantly different ($P > 0.05$).*

	Light trough		Dark trough	
	Pre-inversion	Post-inversion	Pre-inversion	Post-inversion
Hannaea arcus	4.36	1.07	0.92	0.61
Diatoma tenue	3.03	1.80	1.17	0.79
Fragilaria vaucheriae	3.86	1.69	1.59	0.47
Achnanthes minutissima	1.43	1.09	0.97	1.28

Diurnal changes in periphytic diatom buoyancy could explain selective egress from the dark trough only if changes were not endogenous and if sinking rates increased on exposure to light. Anderson and Sweeney (1978) showed that buoyancy changes ceased when cultures were transferred either to conditions of continuous low light or into continuous darkness. Further, in both species of marine diatoms that have been carefully examined, sinking rates increase on exposure to light each day (Anderson and Sweeney 1978, Granata 1987). Although the sinking rate measurements reported here do not establish the presence of a diel rhythm, they indicate that for *H. arcus* and *D. tenue*, sinking rates in light-exposed cells increased during the morning. In addition, for these same species, sinking rates in cells not exposed to morning light declined.

The concensus of most authorities is that diatoms are negatively buoyant (Lund 1959, Smayda 1970, Reynolds 1984). Arguments for negative buoyancy are buttressed by computations using the specific gravity and relative volumes of diatom cell constituents (Smayda 1970, Anderson and Sweeney 1977, Walsby and Reynolds 1980). Nevertheless, it is important to recognize that positive buoyancy is not necessary to effect a selective loss of cells from these highly turbulent lotic systems. All that is required is that buoyancy or form resistance be greater in artificially darkened conditions than in naturally lighted environments. The differential between light and dark sinking rates observed here supports the buoyancy-shift hypothesis as a possible mechanism capable of producing a dark avoidance response in benthic diatoms. Moreover, Granata (1987) showed that the time scale for changes in sinking rate of *Coscinodiscus concinnus* moved from a high to a low light environment is 3–5 h. The dark avoidance response reported here, although initiated within 1 h, was not completed until 2–3 h later.

All the evidence available suggests that the selec-

tive loss of cells from the dark trough is mediated by cellular control of buoyancy or form resistance. If dark avoidance is a widespread phenomenon, it may prove to be an important mechanism contributing to species succession in lotic environments. Light is often considered to be a growth limiting resource in attached algal communities (Hansmann and Phinney 1973, McIntire 1973, Sheath et al. 1986), and light attenuation in thickening algal mats is thought to be a prime factor influencing diatom species succession in attached communities (Patrick 1976, Hudon and Bourget 1983). The demonstration of a dark avoidance response shows that succession in lotic diatom communities can occur in a time frame (hours) much shorter than that required for succession by differential growth (days). Since different species appear to have varying degrees of dark avoidance, each may have its own strategy for dealing with low light environments. The suggestion that some lotic diatoms possess a physiological mechanism which enables them to take advantage of existing water currents and leave an unfavorable environment in search of a more suitable habitat is remarkable considering that araphid, benthic diatoms are usually considered to be non-motile.

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